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PATENT ABSTRACTS OF JAPAN

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(54) PRODUCTION OF SOLID PHASE DETERMINED IN RANGE SPATIALLY AND CLEARLY FOR COUPLING ASSAY

(57)Abstract:
PROBLEM TO BE SOLVED: To produce a solid phase determined in range spatially and clearly for coupling assay.
SOLUTION: A method for coating a solid non-porous support with multilayered coat includes a process (a) applying precoating to the reagent region of a solid support, a process (b) for cleaning the precoated support with an aq. soln., and a process (c) applying second coat containing a receptor molecule capable of being coupled with the precoating layer to the precoated support in a form of a zone, wherein a range is spatially determined on the reagent region and a solid phase is obtained by this method. A solid phase smearing preventing method uses this solid phase and a method for reducing non-specific coupling to the solid phase coated with streptoavidin/avidin uses the solid phase.

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* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The process which is the technique for applying a multilayer coat on a solid non-**** base material, and applies a precoat (under coat) to the reagent field of (a) this solid-state base material, (b) The aforementioned technique containing the process applied with the gestalt of the zone as which the domain was spatially determined [quart / 2nd / containing the process which washes the base material which gave this precoat with a drainage-system solution, and the acceptor molecule which can combine with (c) this precoat] on this reagent field on the given base material in the precoat.
[Claim 2] Technique according to claim 1 that the 2nd aforementioned quart is characterized by being applied with the gestalt with a diameter of 10 micrometers - 10mm of the zone where the domain was defined spatially.
[Claim 3] Technique according to claim 1 or 2 which the aforementioned solid-state base material is pre coated with the 1st joint partner of a specific joint pair, and is characterized by the aforementioned acceptor molecule containing the 2nd joint partner.
[Claim 4] Technique given in any 1 term of claims 1-3 which applies to a base material some zones of the 2nd aforementioned quart where the domain was defined spatially, and is characterized by having the acceptor molecule from which at least two of these zones are different, respectively in there.
[Claim 5] It is the solid phase acquired by technique given in any 1 term of the claims 1-4 which come to contain the precoat given on the solid non-**** base material, and the 2nd quart combined with this precoat with some gestalt of the circular zone where the domain was defined spatially. this -- the acceptor molecule which the 2nd quart has combined with this precoat -- containing -- and -- this -- the aforementioned solid phase characterized by for the diameter of this zone of the 2nd quart being 10 micrometers - 10mm, and the differentiation between each zone being less than 10%
[Claim 6] Use of the solid phase according to claim 5 for detecting the announcer light of one sort or several sorts.
[Claim 7] The process which is the technique for improving the homogeneity of the coating zone where the domain was defined in between solid-state base material absentminded, and applies a precoat on the reagent field of (a) this solid-state base material, (b) The aforementioned technique characterized by including the process applied with the gestalt of the process which washes the base material which gave this precoat with a drainage-system solution, and the zone as which the domain was spatially determined [quart / 2nd / which can combine with (c) this precoat] on this reagent field in this precoat on the base material which gave.
[Claim 8] The coating solution containing an acceptor molecule is applied to the predetermined field to which it was set to the domain of a solid-state base material. Then, it is the method of preventing the smearing of the solid phase as which the domain was determined spatially acquired by processing with a re-coating solution. (a) by application of the thing for which this coating solution is applied so that this acceptor molecule may essentially join together quantitatively in the predetermined field where the domain was defined, and (b) re-coating solution The aforementioned technique of coming to contain at least one of the meanses of preventing [the reunion of the acceptor molecule eluted from the predetermined field where this domain was defined] **.
[Claim 9] Technique according to claim 8 characterized by applying the coating solution containing the acceptor molecule of less than 90% of the concentration of a threshold value concentration needed for occupying completely the predetermined field where the aforementioned domain was defined.
[Claim 10] Can manufacture by technique according to claim 8 or 9, and the coating solution containing an acceptor molecule is applied to the predetermined field of a solid-state base material. Then, the aforementioned solid phase which is manufactured by processing with a re-coating solution, however is characterized by for this acceptor molecule being the solid phase arranged in the zone where the domain was defined spatially, and locating it in the zone where the domain was defined in this space of this acceptor molecule 80% or more.
[Claim 11] The aforementioned technique containing the process which applies the conjugate which is the technique for reducing the un-specific combination to the solid phase which coated streptoavidin or the avidin, and consists of a (a) macromolecule and a biotin, and the process which applies (b) monomer streptoavidin or an avidin to this solid phase.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention relates to the solid phase manufactured by the technique for reducing the un-specific combination to the solid phase which coated the technique, the streptoavidin, or the avidin for preventing the technique for applying a multilayer coat (multilayer coating) to a solid non-**** base material (apply), and the smearing (smearing) of the solid phase (spacially defined) as which the domain was determined spatially, and the technique of this invention.
[0002]
[Description of the Prior Art] The joint assay for measuring announcer lights, such as an immunoassay and a nucleic-acid hybridization assay, is used very broadly. A detection of the announcer light by the joint assay can perform the liquid phase using the uneven examining method make solid phase contact. The solid phase contains the examination zone (test zones) containing the acceptor molecule which an announcer light can combine specifically. Then, an indicator detects combination of an announcer light, for example.
[0003] It is necessary to produce the examination zone of a joint assay using the acceptor molecule of the amount correctly measured by qualitative or the technique of being reproducible for quantitive measurement with the reliability of an announcer light. Differentiation (variation) arises on the detected signal level, and, for the reason, an exact evaluation test result is not obtained by the variation in the size of an examination zone, the obscurity of the boundary of an examination zone, the smearing of an acceptor molecule, the variation of the acceptor density in an examination zone, and the un-unique bonding site.
[0004] These problems usually influence strongly as the examination zone located on solid phase and solid phase becomes small. When each examination zone containing the acceptor molecule from which it is [for detecting a different announcer light] different is mutually close, by the obscurity of the boundary of this examination zone, and/or the smearing of an acceptor molecule, the acceptor molecule to another announcer light may mix to a specific examination zone, and, as a result, the mistaken test result may be brought. This problem is serious in especially the qualitative test for an infective-disease detection. By HIV examination, drawing a conclusion cannot do at all the sample of an incorrect positivity which follows, for example, is produced by the smearing of an acceptor molecule.
[0005] Many examination formats (test formats) are using use of a paper disk or the base material material of the porous material like other porous materials as the base. For example, in the examination format indicated by EP-A-0427984 and EP-A-0063810, an acceptor layer is formed on a porous material by applying an acceptor molecule to a porous material as a solution absorbed by the base material material. A coating field spreads and especially this is dependent on the local fine structure of a base material material with absorption of this coding solution. Therefore, especially the thing for which the uniform examination zone which is reproducible using this technique is produced in a miniaturization (miniaturized) examination format is difficult. Furthermore, in the miniaturization examination format which uses such a porous material base material material as the base, evaluation is difficult. If there are convexo-concave one of the front face of a porous material and an ununiformity, only the optical resolution of a certain limited domain of an examination zone can usually correspond.
[0006] Therefore, the attempt using the base material material which has a non-**** front face instead of a porous material base material material is performed. The U.S. patent of No. 4,591,570 has indicated the immunoassay equipment for the simultaneous detection of some kinds of antigens which used the antibody array, and glass or plastics is used as a base material in there. If this base material material is used, it comes to be able to carry out the considerable reduction of the amount of samples which it is enabled to make the size of the whole examination format fairly small, as a result is needed. However, even if it is the case where a non-**** front face is used, the problem of the smearing of an acceptor molecule is produced, and when the miniaturized test method is used, it is remarkable [especially this]. The cause of a smearing may be what is spread and gone, without controlling the applied coating solution, i.e., the field which is still (regular) a convention of a profile becomes large. In addition, flowing, without controlling the applied coating solution may also happen, and, for this reason, a profile may become irregular (irregular). Finally, the carry-over (loss) of the acceptor molecule to the field of the outside of the zone which has got wet with the coating solution may arise by this smearing.
[0007] Furthermore, many of acceptor molecules like the acceptor molecule of low molecular weight generally used hardly stick to the solid-state base material which has a non-**** front face, or it is adsorbed by this solid-state base material with low repeatability. The drainage-system solution of many acceptor molecules usually used for moreover applying an acceptor molecule

to solid phase contains the surfactant for stabilization, and has prevented partially at least adsorption of the acceptor molecule to the solid-state base material which has a non-**** front face by this.
[0008] A multilayer coat can be given in order to improve the binding affinity of the acceptor molecule to a non-**** front face. However, even when such a coat is used, the variation in a size and bordering obscurity arise in each examination zone, and this poses a remarkable problem in evaluation of a miniaturization examination format especially.
[0009]
[Problem(s) to be Solved by the Invention] Therefore, the purpose of this invention is offering the technique for solving the above-mentioned problem and obtaining the examination zone for joint assays where the domain's was defined clearly spatially.
[0010]
[Means for Solving the Problem] The process at which the above-mentioned purpose is the technique for applying a multilayer coat on a solid non-**** base material, and applies a precoat (under coat) to the reagent field of (a) this solid-state base material by this invention, (b) the process which washes the base material which gave this precoat with a drainage-system solution, and the 2nd quart containing the acceptor molecule which can combine with (c) this precoat It is attained by the above-mentioned technique containing the process applied with the gestalt of the zone as which the domain was spatially determined on this reagent field on the base material which gave the precoat.
[0011] According to this invention, a precoat (under coat;precoating) is first applied to a solid-state non-**** base material. This precoat may be applied over the whole region of a part of front face of the reagent field of a solid-state base material, or the whole front face, or may be applied with the gestalt of a spot (spot). As for a precoat, applying over all fields is desirable. Typically, a precoat is applied to a base material as a drainage-system solution. As long as a precoat enables the 2nd-quart combination, it may consist of what molecule. As for a precoat, it is desirable that the 1st joint partner of a joint pair of high compatibility is included, and streptoavidin, an avidin, a biotin and the analog of these matter, a derivative and conjugate, or an antibody (for example, anti-mouse antibody) is mentioned as such a joint partner, for example. However, it is also possible to apply as a precoat the molecule (for example, molecule containing an amine machine, a sulfide machine, or a silyl machine) designed so that it might join together according to the 2nd quart and covalent bond.
[0012] When applying the acceptor molecule of a drainage-system solution to such a precoat with the gestalt of a small drop, an acceptor molecule is diffused on this precoat from this solution, and it is made to combine with this precoat. However, the applied drop flowed and, as a result, it turns out that differentiation arises in the profile and size of a spot zone. By this, differentiation (variation) arises in the acceptor molecule density in each examination zone, for the reason, differentiation (variation) arises on signal level, and the result which was mistaken after all may be brought.
[0013] By [which should be surprised] introducing a down stream processing (b) (namely, process which washes the pre coated base material with a drainage-system solution) showed especially that a reproducible uniform examination spot comes to be obtained now. Although washing of the pre coated base material may be performed using a pure water, the buffer solution with low ionic strength is used preferably. The drainage-system solution or washing solution used for washing of the pre coated base material can contain the buffer matter, a surfactant, and/or other additives. Fundamentally, as buffer matter, the buffer matter usual [all] or the buffer matter well-known to this contractor is usable. 250 or less mMs of 100 or less mMs of the concentration of the buffer matter which exists in a washing solution are 40 or less mMs most preferably especially preferably. That the best result is obtained about the function by this invention as a penetrant remover is the case where 5 or less mMs of 10 or less mMs of buffer matter exist by the concentration of 2 or less mMs most preferably. Some buffer matter and those desirable concentration domains (C1), especially desirable concentration domains (C2), and the most desirable concentration domains (C3) are shown in Table 1.
[0014]
[Table 1]

緩衝物質	C_1 [mM]	C_2 [mM]	C_3 [mM]
リン酸塩	≦40	≦5	≦2
炭酸塩	≦40	≦5	≦2
Mes/Tris	≦100	≦10	≦5
Hepes/Tris	≦100	≦10	≦6

[0015] In order to optimize the technique of this invention further, it is possible to choose the buffer which guarantees the optimum reaction condition according to the modality of interaction which combines an acceptor molecule with a precoat. For example, in the precoat containing streptoavidin, the pH domain of 5-9 is the optimum.
[0016] According to this invention, the combination of the buffer containing a volatile component like an acetic acid or a formic acid is also usable. However, in such combination of a buffer, after a volatile component's volatilizing and becoming the residue of a buffer, pH on a precoat may be changed greatly. It is because only one component of a buffer volatilizes and other components remain in a front face. Therefore, it is desirable to use the combination of the buffer which consists of only a volatile component or a nonvolatile component.
[0017] Again, in addition to the buffer matter, the washing solution used by this invention can be replaced with the buffer matter, and can contain a surfactant. It assists that adding a surfactant in a washing solution removes a washing solution from a precoat, without leaving the residue substantially. As for especially a washing solution, it is desirable to contain a surfactant by the concentration below 0.005% (vol/vol) most preferably below 0.01% (vol/vol) still preferably below 0.02% (vol/vol) below 0.05

(the volume to full capacity ******)% (vol/vol). For the concentration of the surfactant in a washing solution, it is in the case below 0.001% (vol/vol) most preferably that the best result is obtained below 0.002% (vol/vol) still preferably below 0.003% (vol/vol). About some desirable surfactants, the desirable concentration C1, the desirable concentration C2, and the most desirable concentration C3 are especially shown in Table 2.

[0018]

[Table 2]

界面活性剤	C_1 [%(vol/vol)]	C_2 [%(vol/vol)]	C_3 [%(vol/vol)]
Tween 20	≦0.01	≦0.005	≦0.003
n-オクチルグルコシド	≦0.02	≦0.01	≦0.003
Chaps	≦0.01	≦0.005	≦0.003
Brj 35	≦0.005	≦0.002	≦0.001

[0019] All surfactants are fundamentally suitable to remove a washing solution, without leaving the residue substantially. It can be determined by the preliminary experiment of 2-3 according to the modality and technical conditions (for example, format of a reagent base material) of a coat whether a certain surfactant is suitable for a certain examination format if it is this contractor. Especially, the liquid applied behind must flow with a surfactant, or you have to pay attention so that only a few will not spread. From this ground, especially (related especially with operating concentration) when using a surfactant SDS, you have to pay attention.

[0020] The washing solution used by this invention can also contain again other additives which have an advantageous effect to the technique of this invention. The additive which has a stabilization effect to a precoat especially can be used. It is enabled to open a time interval in a manufacture process by this between pre coating of a reagent base material, and an application of the specific coat for each joint assays. A saccharide, salts, and a blocking reagent are mentioned as an example of such other additives. The desirable concentration C1 about these matter, the desirable concentration C2, and the most desirable concentration C3 are especially shown in Table 3.

[0021]

[Table 3]

物質	C_1 [% (wt/vol)]	C_2 [% (wt/vol)]	C_3 [% (wt/vol)]
糖類	≦0.5	≦0.1	≦0.05
塩類	≦0.5	≦0.1	≦0.05
ブロッキング試薬	≦0.2	≦0.02	≦0.005

[0022] The molecular weight of a desirable saccharide is the saccharide (for example, cane sugar) of 500 or less low molecular weight especially preferably 1000 or less. The sugar oligomer of the low molecular weight containing one, two, or three sugar units is the most desirable. The salts used preferably are salts which seldom form a crystal like a disodium tartrate and a dihydrate. As a blocking reagent, the protein of the low molecular weight like a peptone is used preferably.

[0023] The concentration of each matter in a washing solution can be adjusted according to the specific requirements for an examination, such as a modality of coat, and a size of an examination format. In order to attain suction which makes the amount of the possible residue the minimum in the case of the reagent base material which follows, for example, has a comparatively big reaction field (diameter:> 1cm), it is desirable to use the washing solution containing a pure water or the comparatively low-concentration above-mentioned matter (a buffer, a surfactant, other additives).

[0024] As for a drainage-system solution, it is desirable to pass quickly on a precoat, to remove completely next (for example, suction), and to make a precoat into a dryness. It is desirable to use the precoat which has a canal-property.

[0025] Next, the 2nd quart is applied to the washed precoat. It is applied on a reagent field as a zone where the domain was defined spatially including this acceptor molecule that can combine the 2nd quart with a precoat. As for an acceptor molecule, it is desirable to contain the 2nd joint partner of a joint pair who can do the interaction of for example, an immunological reaction, streptoavidin / avidin interaction, etc. to the 1st joint partner of a joint pair applied as a precoat by high compatibility, or can join together by covalent bond. It is got blocked, for example, streptoavidin or an avidin may be applied as a precoat, and an acceptor molecule contains a biotin component in that case. As for an acceptor molecule, it is desirable to make it combine with a precoat according to the equilibrium constant KM>=108 l/mol interaction of high compatibility. By the 2nd-quart application to the washed precoat, the examination zone of reproducible uniform profile and size can be obtained.

[0026] This effect becomes still clear with an attached drawing. Drawing 1 shows the miniaturization examination zone obtained by the conventional method of not performing a washing process. Drawing 2 shows the miniaturization examination zone obtained by the technique of this invention.

[0027] Drawing 3 is a thing explaining the effect of a washing process. a among drawing 3 What put water into Enzymun test tube (polystyrene spool which is not coated) which is not coated is shown. Enzymun test tube (the polystyrene spool which coated streptoavidin --) with which b coated streptoavidin What put water into Boehringer Mannheim, order-number No.1144553, and "Enzymun-Test Streptavidin Tube" is shown. After coating c with streptoavidin, it shows what put water into Enzymun test tube

washed and dried.
[0028] It is proved clearly that the spot profile of the advantage attained according to a washing process, i.e., the uniform examination zone which has repeatability on a reagent field, is obtained by comparison of the examination zone shown in the drawing 1 and the drawing 2 .
[0029] When water is put into Enzymun test tube which is not coated as shown in a of drawing 3 , a completely level water-air interface is formed. This means that **** of the wall surface by water is small. On the other hand, when water is put into Enzymun test tube pre coated with streptoavidin, the water-air interface which curved greatly as shown in b of drawing 3 arises. By pre coating, **** of a wall surface becomes large. When washing ****** of the test tube first pre coated with streptoavidin is violently carried out with a drainage-system solution, as shown in c of drawing 3 , a level water-air interface arises. A precoat shows a canal-property after a washing process and, for this reason, a wall surface does not get wet slightly merely in water.
[0030] According to this invention, the non-**** material of all solid-states can be used as a solid non-**** base material. As for this base material, what consists of tic [a metal, glass or / a press] preferably, and especially consists of polystyrene is desirable.
[0031] As for the 2nd quart, it is desirable to apply with the gestalt with a diameter of 10 micrometers - 10mm of the zone where the domain was defined spatially. Since especially the technique of this invention is suitable for production of a miniaturization examination format, as for the 2nd quart, it is desirable to apply preferably especially with the gestalt with a diameter of 20 micrometers - 200 micrometers of the zone where the domain was defined spatially the diameter of 10 micrometers - 500 micrometers. It is possible to perform the 2nd-quart application using well-known technique. It is [the 2nd quart] convenient to apply by using for example, a liquid jet process (liquid jet process) as a drainage-system solution with the gestalt of the minute drop of volume l of 0.00001-1micro. However, according to this invention, it is also possible to coat a microtiter plate and a big reaction container like a cuvette.
[0032] The whole field of the reagent field of a solid-state base material is coated, and it is desirable to apply the 2nd quart to the precoat of this whole field with the gestalt of the examination zone where the domain was defined spatially after that. However, it is also possible to apply a precoat beforehand with the gestalt of a spot. However, since the 2nd quart must be correctly arranged on this pre coated zone in this case, it will take time further.
[0033] Since the technique of this invention is reproducible, it is enabled to offer this examination system of the gestalt miniaturized so that the considerable reduction of the amount and analysis time of the examination system containing some identities or different examination zones, especially the announcer light needed could be carried out. Therefore, it is desirable to apply to a base material the zone which is the 2nd quart as which the domain was determined spatially [some]. All of these zones may contain the same acceptor molecule. In this case, it is possible to examine simultaneously two or more sorts of different samples on one examination base material. When the acceptor molecule from which at least two of these zones are different is included, it is possible to measure the announcer light of several sorts simultaneously in one sample. By such system, the thing for which the examination zone where the domain was defined clearly spatially is produced, and especially the thing for which every smearing of an acceptor molecule is prevented are important. It is enabled to ensure not bringing the result which contamination of the neighboring zone by the thing for which surely the announcer light of the purpose combinable with a specific examination zone is measured, and the acceptor molecule of other examination zones mistook only in such a case.
[0034] The precoat which could acquire still another theme of this invention by the above-mentioned technique, and was given on the solid-state base material, the solid phase which comes to contain the 2nd quart combined with this precoat with some gestalt of the circular zone where the domain was defined spatially -- it is -- this -- containing the acceptor molecule which the 2nd quart has combined with this precoat -- and -- this -- it is the above-mentioned solid phase characterized by for the diameter of the zone of the 2nd quart being 10 micrometers - 10mm, and the differentiation between each zone being less than 2.5% especially preferably less than 5% preferably less than 10% As for this solid phase, it is desirable that the diameter of an examination zone especially uses 10-500 micrometers by the 20-200-micrometer miniaturization examination system preferably.
[0035] Furthermore, it includes that such solid phase is used for this invention in joint assays, such as a thing to use for a detection of the announcer light of one sort or several sorts especially an immunoassay, and a nucleic-acid hybridization assay.
[0036] The process which still another theme of this invention is the technique for improving the homogeneity of a coating zone as which the domain was determined in between non-**** base material absentminded on a solid-state base material, and applies a precoat on the reagent field of (a) this solid-state base material, (b) the process which washes the base material which gave this precoat with a drainage-system solution, and the 2nd quart which can combine with (c) this precoat It is the above-mentioned technique characterized by including the process applied with the gestalt of the zone as which the domain was spatially determined on this reagent field on the base material which gave this precoat, and **.
[0037] The problem produced frequently [in case the solid phase for joint assays is manufactured and/or used] is the smearing of the acceptor molecule to the outside of the zone which is made into the purpose at the time of applying a re-coating solution and where the domain was defined spatially.
[0038] The 2nd mode of this invention therefore, the coating solution containing an acceptor molecule Are obtained by applying and processing to the predetermined field to which the domain of a solid-state base material was defined with a re-coating solution continuously. This coating solution is applied so that it may be the technique of preventing the smearing of the solid phase as which the domain was determined spatially and (a) this acceptor molecule may essentially join together quantitatively in the predetermined field where the domain was defined, And it is related with the above-mentioned technique of coming to contain at least one of the meanses of preventing [the reunion of the acceptor molecule eluted by application of (b) after-treatment solution (re-coating solution) from the predetermined field where this domain was defined] **.

[0039] Especially, by [which should be surprised] using at least one of the meanses of these, the smearing of the solid phase as which the domain was determined spatially could be prevented, and it became clear that the examination zone which has the profile and size which are reproducible as the result and where the domain was defined clearly is obtained. This technique is monolayer coating] usable also to the multilayer coating tip.
[0040] An acceptor molecule may usually be applied to a solid-state base material with the gestalt of a drop small as a drainage-system solution, and this base material may be pre coated by the case. Next, an acceptor molecule is diffused from this solution to this solid-state base material or a precoat, and is combined with this. This joint interaction may be an interaction of high compatibility like an immunological reaction, or streptoavidin / avidin-biotin interaction, or may be covalent bond. By the usual solid phase system of the conventional technique, when the experiment for analyzing for example, an announcer light is conducted according to the process which continues after a coat application, or when a re-coating solution is applied, the elution of the molecule which has not been combined pertinently uncombined from this solid phase happens.
[0041] The solid phase coated specifically is processed with the re-coating solution which has the function which blocks an un-specific bonding site and usually stabilizes an acceptor molecule to the influence from the exterior. The ground this is needed is that the examination zone on the reagent field of solid phase is not usually occupied 100% completely. However, the free fraction which remains on the base material or the precoat corresponds to the un-unique bonding site which other sample components other than the target announcer light can combine.
[0042] Furthermore, the coat of an acceptor molecule usually also contains the trauma molecule of slight proportion, and other sample components other than the target announcer light can combine it there in un-unique. Such a site is blocked by applying the re-coating solution containing for example, inactive protein, and, in this way, a sample component can combine it no longer later during analysis. Furthermore, a re-coating solution is useful also to stabilizing an acceptor molecule or coating to the influence of external (for example, temperature and humidity) in many cases. For example, in many embodiments of an analysis system like a microtiter plate or ES tube, operation in which a re-coating solution achieves a blocking operation and a stabilization effect exists.

[0043] Although the re-coating solution needs to be used for many examination formats, this is accompanied by the smearing of the acceptor molecule applied previously simultaneously. If a re-coating solution is applied, the elution of the acceptor molecule which has not been combined pertinently uncombined will happen. The regrettable smearing of the solid phase as which the domain was determined especially spatially beforehand combinable [these elution acceptor molecules] with the still combinable site (accessible sites) of a solid-state base material or a precoat therefore arises. An acceptor molecule is because it can adhere besides the 1st application section. Such a smearing is a serious fault when the zone of some containing an acceptor molecule especially different, respectively where the domain was defined spatially is applied to a base material. Since elution of an acceptor molecule and combination of the elution acceptor molecule to other sites of solid phase imitate mixture of the acceptor in an examination zone and may come, they become unable to obtain a clear analysis result. This problem is produced especially in a miniaturization examination format. Also in the examination for which a re-coating solution is not used at all, the same problem arises at the time of the last which applied the sample solution.
[0044] It became clear that it is possible to prevent the smearing of the solid phase as which the domain was determined spatially by now applying the coating solution which should be surprised and which especially essentially causes combination of a quantitive acceptor molecule in a predetermined zone. Since an acceptor molecule joins together about 100%, the elution of this acceptor molecule can be prevented. Many especially more preferably [it is desirable and] than 90%, from 95%, there are many acceptor molecules and they are combined. [than 99%] [more / it is the most desirable and] As for a quantitive combination of an acceptor molecule, it is essentially desirable to be attained by using the coating solution which has acceptor molecule concentration lower than sufficient threshold value concentration to occupy a predetermined zone completely. When a coating solution contains an acceptor molecule fewer than the amount which can be combined with an examination zone, all the applied acceptor molecules are combined with an examination zone, it becomes behind and the uncombined acceptor molecule or the acceptor molecule which has not been combined pertinently which may be eluted (for example, re-coating solution) does not exist. The threshold value concentration about various kinds of acceptor molecules is shown in Table 4.
[0045]
[Table 4]
表4：各種コーティング分子の限界濃度

受容体分子	直径 10μm	直径 100μm	直径 1 mm
抗体	2.5 mg/ml	250μg/ml	25μg/ml
抗原（例えばp24）	600μg/ml	60μg/ml	6 μg/ml
ペプチド(MW 3000)	150μg/ml	15μg/ml	1.5μg/ml

[0046] It is desirable to use the coating solution containing the acceptor molecule of concentration (especially [Less than 50% / Preferably / of a threshold value concentration] good **** less than 25%) lower than a threshold value concentration.
[0047] furthermore, not the acceptor molecule combined powerfully, for example, a monomer antibody, but bridge formation -- it is desirable to use an antibody It is possible to prevent a smearing by making an antibody construct a bridge and forming the polymer (polymer) of molecular weight bigger than 1,200,000 Dalton especially. The protein with which the ground constructed

the bridge highly is because it became clear to combine with a solid-state base material (especially plastics front face) very well. The tBSA conjugate like the recombination field HBc antigen which carries out self-flocculation and forms the macromolecule of the domain of several 1 million Dalton and tBSA-biotin, tBSA-streptoavidin, or tBSA-anti--TSH antibody is especially used preferably as an acceptor molecule. It became clear the thing which an acceptor molecule combines about 100% in all cases, therefore that the smearing of an acceptor molecule does not arise at all on the outside of the zone made into the purpose.
[0048] You may apply an acceptor molecule to the precoat which may apply to a solid-state base material directly, or was applied to the solid-state base material. It is desirable to use the acceptor molecule which has 108 l/mol high joint compatibility at least to this precoat in the case of a precoat.
[0049] Furthermore, that it can prevent by adding a elution retarder in a coating solution made clear the smearing of the solid phase as which the domain was determined spatially. A elution retarder forms a coat (paint film) on an examination zone, after a coating solution dries, and it is understood to be the matter by which the direct elution of the uncombined acceptor molecule at the time of adding a re-coating solution or a sample solution or the acceptor molecule which has not been combined pertinently is prevented or delayed. a suitable elution retarder is like cane sugar, PVP (polyvinyl pyrrolidone), or a dextran -- molecular weight is the saccharide of 10,000 to 100,000 Dalton preferably Other suitable elution retarders are gelatin and a cellulosic, and when an acceptor molecule reacts with the precoat applied previously, especially being used preferably is a methyl cellulose. Addition of a elution retarder delays the elution of an uncombined acceptor molecule until it is also probed that it is blocked by the means with all the free sites suitable at least on a solid-state base material, and a elution acceptor molecule already joins together and it is lost.

[0050] In the 2nd mode, the reunion of the acceptor molecule eluted from the predetermined field is prevented by applying a re-coating solution. This is preferably attained by adding the blocking matter in a re-coating solution. In this case, it is important to ensure to restrict and to join together quickly with the possible blocking matter. This can be preferably attained 0.5% of the weight or more by adding 1% of the weight or more of the high-concentration blocking matter (bovine serum albumin), for example, 1% of the weight of BSA, protein C, casein, etc. in a re-coating solution. Still suitable blocking matter is a peptone, cow IgG, lagomorph IgG, a collagen, gelatin, a polyethylene glycol, and a surfactant (for example, Tween 20).
[0051] The joint speed of the blocking matter (especially blocking protein) is improvable again with a suitable buffer additive like MgCl2 3%. In applying to a precoat rather than applying a coating solution to a solid-state base material directly, it chooses the blocking matter which reacts specifically with this precoat. In addition to it instead of [like blocking protein] the un-specific blocking matter, the blocking effect is further improvable by using the combinable specific blocking matter for a precoat specifically. In this case, the solution containing a biotin-ized acceptor molecule is applied for example, to a streptoavidin precoat. A biotin solution is applied to this after xeransis, and a still free streptoavidin bonding site is still saturated.
[0052] It is also possible to prevent the smearing of the solid phase as which the domain was spatially determined by applying a re-coating solution for a short time so that the eluted acceptor molecule may be thinned powerfully immediately according to this invention finally. the longest time about this -- usually -- they are 50 mses especially preferably 250 mses preferably 500 mses In this case, it is advantageous that quick mixture is ensured and the field where a elution acceptor molecule becomes high concentration locally in a re-coating solution is made not to be generated. Efficient mixture can attain for example, a re-coating solution by making certainly into the minimum the contact time of the possible re-coating solution which restricts, applies by the early rate of flow, and is possible, and an examination zone.
[0053] Especially, it uses preferably combining some in the above-mentioned means, and the eluted acceptor molecule is made not to join out of the target examination zone together. A elution retarder is added in a coating solution, the blocking matter is added in a re-coating solution, and it is clear that especially the thing that quick mixture of a re-coating solution is ensured simultaneously with it is advantageous.
[0054] Since especially this technique is advantageous in the miniaturization examination format, as for the solid phase as which the domain was determined spatially, it is desirable that 10-500 micrometers of diameters are 20-200 micrometers especially preferably.
[0055] this invention relates to the solid phase acquired by the above-mentioned technique for preventing the smearing of the solid phase as which the domain was determined spatially again. this solid phase It is manufactured by applying and processing the coating solution containing an acceptor molecule to the predetermined field of a solid-state base material with a re-coating solution continuously, and sets there. This acceptor molecule is arranged in the zone where the domain was defined spatially, and it is characterized by existing 90% or more 80% or more in the zone of this acceptor molecule where the domain was defined especially in [it is / 95% or more of / especially desirable, and] this space. Such solid phase makes possible the analysis result in which a reliability has repeatability highly. This kind of solid phase is usable to the detection of the announcer light of one sort or several sorts.
[0056] Many another causes of a result that the mistake was able to be made in in a joint assay are combination of sample components other than the announcer light of the purpose to the solid phase which coated an un-unique combination, i.e., streptoavidin, or the avidin. Therefore, this invention is the technique for reducing again the un-unique combination to the solid phase which coated streptoavidin or the avidin, and relates to the above-mentioned technique containing the process which applies the conjugate which consists of a (a) macromolecule and a biotin, and the process which applies (b) monomer streptoavidin or an avidin to solid phase.
[0057] It made this technique clear especially that the considerable reduction of the un-unique combination to the solid phase which should be surprised and which coated streptoavidin or the avidin can be carried out. By using monomer streptoavidin, the

considerable reduction of the un-unique combination of the conjugate to solid phase and other sample components is carried out especially.
[0058] The conjugate which consists of the macromolecule and biotin like tBSA preferably is applied to a solid-state base material with the gestalt of a minute drop as a drainage-system solution. In this case, it is desirable that a macromolecule and a biotin use 1:1-1:3, and the conjugate that becomes by the proportion of 1:1-1:2 preferably especially. Thus, if the coating protein of the few amount of biotin-ized theory is used, it will be enabled to reduce an un-unique combination of especially conjugate further much more.
[0059] The diameter of solid phase is especially preferably [especially] suitable to the miniaturization examination format of 5mm or less for the technique of this invention for reducing the un-specific combination to the solid phase which coated streptoavidin or the avidin 10mm or less preferably again. Further, another theme is solid phase acquired by the above-mentioned technique of this invention, and this is characterized by having the layer of the monomer streptoavidin given on the precoat of the conjugate which consists of a macromolecule and a biotin, and this precoat, or an avidin. Such solid phase [especially] is used for a detection of an announcer light. The following examples explain this invention further.
[0060]
[Example] The production examination zone of the spot to the front face which coated [example 1] streptoavidin was coated with the coating solution containing amount compound BSA[of macromolecules]-streptoavidin (particle size : about 100nm). The coating solution was attracted after the reaction time for 15 minutes, and the examination zone was blocked with NaCl, 0.2% cane sugar, and the solution that contains BSA 0.05% 0.05%. In the examination zone shown in drawing 1 , on the other hand, using the drainage-system medium, after incubating for 5 minutes, the suction xeransis of the precoat was only carried out, it carried out, and the suction xeransis of the washing process by this invention was carried out continuously in the examination zone shown in drawing 2 .
[0061] [Example 2] 3.7g Tris [a tris (hydroxymethyl) aminomethane] and 4.75g MES (2-morpholino ethane sulfonic acid) were melted in 5l. water. 5ml of this washing buffer solution was poured and washed in the pre coated reaction container (nominal capacity:50microl). Then, the suction xeransis of this reaction container was carried out using the suction needle (aspiration needles) of one piece or some. By applying the 2nd quart on this washing and the dry precoat, an examination zone which is shown in drawing 2 and where the domain was defined narrowly spatially was obtained.
[0062] The [example 3] streptoavidin precoat was applied on the solid-state base material. On this coat, the coating solution containing a mono-biotin-ized antibody was applied with the gestalt of a small drop. After drying this drop, the solution which contains a 3mg [/ml] biotin in 50mM K2HPO4 was applied to this. This biotin solution was removed after several seconds, the solution which contains BSA (bovine serum albumin) and 2% cane sugar 1% was applied, and this was also removed after several seconds. Then, this system was dried. Thus, the solid phase without the smearing of the acceptor molecule in the outside of the target reaction field as which the domain was determined clearly spatially was acquired.
[0063] [Example 4] It investigated about the un-specific combination on various kinds of streptoavidin solid phase. A result is shown in Table 5.
[0064]
[Table 5]

コートの種類	TSH コンジュゲート	p24 コンジュゲート	ヒトIgG
tBSA－ストレプトアビジン	24	31	105
tBSA－ビオチン ＋ 多量体ストレプトアビジン	3	71	63
tBSA－ビオチン ＋ 単量体ストレプトアビジン	0	4	38

[0065] An un-unique combination is considerably reduced with the combination of the conjugate and monomer streptoavidin by this invention so that clearly from the above.
[0066] The same peptide of [example 5] direct coating **** [some] was combined with BSA, and the poly-hapten was obtained. This poly-hapten was melted by 50microg [/ml] concentration into the buffer solution containing 5mM Mes, 5mM Tris, and 1% cane sugar. Then, the drop of volume 150pl was applied to the base material made from polystyrene, and it incubated for about 5 seconds, and dried for about 60 seconds. Then, the blocking solution which contains BSA and 2% cane sugar 1% was applied, and it incubated for 10 seconds. Then, this solution was attracted and the blocking solution which contains BSA and 2% cane sugar 1% was added. Then, this solution was attracted and it dried.
[0067] Pre coating **** of [example 6] streptoavidin and the biotin-ized macromolecule (for example, heat flocculation BSA) were applied to the base material made from polystyrene (nominal capacity:50microl) by 100microg [/ml] (inside of PBS) concentration, and it incubated for 5 minutes. Then, this was attracted and the streptoavidin solution of 100microg [/ml] (inside of PBS) concentration was added. After incubating for 5 minutes, the washing process was performed, and the suction xeransis of this solution was carried out continuously.
[0068] Various kinds of biotin-ized DNA usurpation probes (18-mer) were melted in 5mM Mes, 5mM Tris, 1% cane sugar, and the buffer solution that contains 0.5mg /of BSAs ml by the concentration of 1microM. Then, the drop of volume abbreviation

150pl was applied to the configuration of an array at the base material made from polystyrene. After having incubated for about 5 seconds and drying for about 60 seconds, in the 50mM phosphoric-acid 2 potassium, the solution containing a 3mg [/ml] biotin was added, and it incubated for 2 seconds. Then, this solution was attracted and the blocking solution which contains BSA and 2% cane sugar 1% was added, and this solution was attracted and it dried.
[0069] Pre coating PAB<mouse-Fcgamma> S IgG by the [example 7] **-mouse antibody was melted in the buffer solution (pH 7.4) which contains 40mM potassium phosphate in 0.9% sodium chloride by 30microg [/ml] concentration. This solution was added to the reaction container (nominal capacity:50microl). After having carried out the incubation for 15 minutes and drawing in continuously, the blocking solution which contains BSA and 2% cane sugar 1% was added. After incubating for 5 minutes, the washing process was performed according to this invention, and the suction xeransis of this solution was carried out continuously.

[0070] Fusion protein (mouse-Fcgamma+CD28) was melted in 5mM Mes, 5mM Tris, 1% cane sugar, and the buffer solution that contains 0.5mg /of BSAs ml by 100microg [/ml] concentration. It applied to the base material made from polystyrene which the above [the drop of volume abbreviation 150pl] coated, and incubated for about 5 seconds. After drying for about 60 seconds, 50mM potassium phosphate buffer solution (pH 7.4), the 100microg [/ml] mouse IgG, and the blocking solution that contains 1mg /of BSAs ml were added. After incubating for 10 seconds, this solution was attracted and the blocking solution which contains BSA and 2% cane sugar 1% was added, and this solution was attracted and it dried.
[0071] The macromolecule with a particle size [containing [example 8] universal streptoavidin examination zone BSA and streptoavidin] of about 100nm was melted in the solution containing 20mM phosphate buffer solution (pH 7.4) and 1% cane sugar by 500microg [/ml] concentration. The drop of volume abbreviation 150pl was applied to the base material made from polystyrene, and it incubated for about 5 seconds, and dried for about 60 seconds. Then, it added, and it incubated for about 10 seconds and the solution which contains BSA and 2% cane sugar 1% was attracted. Next, it added, and it incubated for 20 minutes and the solution which contains BSA, 2% cane sugar, and 1microg [/ml] biotin-ized anti--TSH antibody 1% was attracted. Finally the blocking solution which contains BSA and 2% cane sugar 1% was added and attracted, and it dried. Consequently, very precise miniaturization TSH specific solid phase was acquired.
[0072] By 100microg [/ml] (inside of PBS) concentration, it applied to the base material made from polystyrene (nominal capacity:50microl), and it incubated for 5 minutes and the examination zone biotin-ized macromolecule for [example 9] **-German measles antibodies (for example, heat flocculation BSA) was attracted. Then, the streptoavidin solution of 100microg [/ml] (inside of PBS) concentration was added. After incubating for 5 minutes, the washing process was performed according to this invention, and the suction xeransis of this solution was carried out continuously.
[0073] The biotin-ized **-German measles antibody was melted in the buffer solution containing 5mM Mes, 5mM Tris, 1% cane sugar, and 0.5mg [/ml] BSA by 50microg [/ml] concentration. The drop of volume abbreviation 150pl was applied to the above-mentioned base material. It incubated for about 5 seconds and this was dried for about 60 seconds. Then, in the 50mM phosphoric-acid 2 potassium, the solution containing 3mg [/ml] biotin was added, it incubated for about 2 seconds, and this solution was attracted. Next, 0.1%, it added, and it incubated for 20 minutes and Tween 20, and BSA and the solution which contains a 3U/ml virus (rubella virus) lysate in PBS were attracted 1%. Then, the blocking solution which contains BSA and 2% cane sugar 1% was added and attracted, and it dried.

[Translation done.]

* NOTICES *

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Field

[The technical field to which invention belongs] this invention relates to the solid phase manufactured by the technique for reducing the un-specific combination to the solid phase which coated the technique, the streptoavidin, or the avidin for preventing the technique for applying a multilayer coat (multilayer coating) to a solid non-**** base material (apply), and the smearing (smearing) of the solid phase (spacially defined) as which the domain was determined spatially, and the technique of this invention.

[Translation done.]

Technique

[Description of the Prior Art] The joint assay for measuring announcer lights, such as an immunoassay and a nucleic-acid hybridization assay, is used very broadly. A detection of the announcer light by the joint assay can perform the liquid phase using the uneven examining method make solid phase contact. The solid phase contains the examination zone (test zones) containing the acceptor molecule which an announcer light can combine specifically. Then, an indicator detects combination of an announcer light, for example.

[0003] It is necessary to produce the examination zone of a joint assay using the acceptor molecule of the amount correctly measured by qualitative or the technique of being reproducible for quantitive measurement with the reliability of an announcer light. Differentiation (variation) arises on the detected signal level, and, for the reason, an exact evaluation test result is not obtained by the variation in the size of an examination zone, the obscurity of the boundary of an examination zone, the smearing of an acceptor molecule, the variation of the acceptor density in an examination zone, and the un-unique bonding site.

[0004] These problems usually influence strongly as the examination zone located on solid phase and solid phase becomes small. When each examination zone containing the acceptor molecule from which it is [for detecting a different announcer light] different is mutually close, by the obscurity of the boundary of this examination zone, and/or the smearing of an acceptor molecule, the acceptor molecule to another announcer light may mix to a specific examination zone, and, as a result, the mistaken test result may be brought. This problem is serious in especially the qualitative test for an infective-disease detection. By HIV examination, drawing a conclusion cannot do at all the sample of an incorrect positivity which follows, for example, is produced by the smearing of an acceptor molecule.

[0005] Many examination formats (test formats) are using use of a paper disk or the base material material of the porous material like other porous materials as the base. For example, in the examination format indicated by EP-A-0427984 and EP-A-0063810, an acceptor layer is formed on a porous material by applying an acceptor molecule to a porous material as a solution absorbed by the base material material. A coating field spreads and especially this is dependent on the local fine structure of a base material material with absorption of this coding solution. Therefore, especially the thing for which the uniform examination zone which is reproducible using this technique is produced in a miniaturization (miniaturized) examination format is difficult. Furthermore, in the miniaturization examination format which uses such a porous material base material material as the base, evaluation is difficult. If there are convexo-concave one of the front face of a porous material and an ununiformity, only the optical resolution of a certain limited domain of an examination zone can usually correspond.

[0006] Therefore, the attempt using the base material material which has a non-**** front face instead of a porous material base material material is performed. The U.S. patent of No. 4,591,570 has indicated the immunoassay equipment for the simultaneous detection of some kinds of antigens which used the antibody array, and glass or plastics is used as a base material in there. If this base material material is used, it comes to be able to carry out the considerable reduction of the amount of samples which it is enabled to make the size of the whole examination format fairly small, as a result is needed. However, even if it is the case where a non-**** front face is used, the problem of the smearing of an acceptor molecule is produced, and when the miniaturized test method is used, it is remarkable [especially this]. The cause of a smearing may be what is spread and gone, without controlling the applied coating solution, i.e., the field which is still (regular) a convention of a profile becomes large. In addition, flowing, without controlling the applied coating solution may also happen, and, for this reason, a profile may become irregular (irregular). Finally, the carry-over (loss) of the acceptor molecule to the field of the outside of the zone which has got wet with the coating solution may arise by this smearing.

[0007] Furthermore, many of acceptor molecules like the acceptor molecule of low molecular weight generally used hardly stick to the solid-state base material which has a non-**** front face, or it is adsorbed by this solid-state base material with low repeatability. The drainage-system solution of many acceptor molecules usually used for moreover applying an acceptor molecule to solid phase contains the surfactant for stabilization, and has prevented partially at least adsorption of the acceptor molecule to the solid-state base material which has a non-**** front face by this.

[0008] A multilayer coat can be given in order to improve the binding affinity of the acceptor molecule to a non-**** front face. However, even when such a coat is used, the variation in a size and bordering obscurity arise in each examination zone, and this poses a remarkable problem in evaluation of a miniaturization examination format especially.

[Translation done.]

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] Therefore, the purpose of this invention is offering the technique for solving the above-mentioned problem and obtaining the examination zone for joint assays where the domain's was defined clearly spatially.

[Translation done.]

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MEANS

[Means for Solving the Problem] The process at which the above-mentioned purpose is the technique for applying a multilayer coat on a solid non-**** base material, and applies a precoat (under coat) to the reagent field of (a) this solid-state base material by this invention, (b) the process which washes the base material which gave this precoat with a drainage-system solution, and the 2nd quart containing the acceptor molecule which can combine with (c) this precoat It is attained by the above-mentioned technique containing the process applied with the gestalt of the zone as which the domain was spatially determined on this reagent field on the base material which gave the precoat.

[0011] According to this invention, a precoat (under coat;precoating) is first applied to a solid-state non-**** base material. This precoat may be applied over the whole region of a part of front face of the reagent field of a solid-state base material, or the whole front face, or may be applied with the gestalt of a spot (spot). As for a precoat, applying over all fields is desirable. Typically, a precoat is applied to a base material as a drainage-system solution. As long as a precoat enables the 2nd-quart combination, it may consist of what molecule. As for a precoat, it is desirable that the 1st joint partner of a joint pair of high compatibility is included, and streptoavidin, an avidin, a biotin and the analog of these matter, a derivative and conjugate, or an antibody (for example, anti-mouse antibody) is mentioned as such a joint partner, for example. However, it is also possible to apply as a precoat the molecule (for example, molecule containing an amine machine, a sulfide machine, or a silyl machine) designed so that it might join together according to the 2nd quart and covalent bond.

[0012] When applying the acceptor molecule of a drainage-system solution to such a precoat with the gestalt of a small drop, an acceptor molecule is diffused on this precoat from this solution, and it is made to combine with this precoat. However, the applied drop flowed and, as a result, it turns out that differentiation arises in the profile and size of a spot zone. By this, differentiation (variation) arises in the acceptor molecule density in each examination zone, for the reason, differentiation (variation) arises on signal level, and the result which was mistaken after all may be brought.

[0013] By [which should be surprised] introducing a down stream processing (b) (namely, process which washes the pre coated base material with a drainage-system solution) showed especially that a reproducible uniform examination spot comes to be obtained now. Although washing of the pre coated base material may be performed using a pure water, the buffer solution with low ionic strength is used preferably. The drainage-system solution or washing solution used for washing of the pre coated base material can contain the buffer matter, a surfactant, and/or other additives. Fundamentally, as buffer matter, the buffer matter usual [all] or the buffer matter well-known to this contractor is usable. 250 or less mMs of 100 or less mMs of the concentration of the buffer matter which exists in a washing solution are 40 or less mMs most preferably especially preferably. That the best result is obtained about the function by this invention as a penetrant remover is the case where 5 or less mMs of 10 or less mMs of buffer matter exist by the concentration of 2 or less mMs most preferably. Some buffer matter and those desirable concentration domains (C1), especially desirable concentration domains (C2), and the most desirable concentration domains (C3) are shown in Table 1.

[0014]

[Table 1]

緩衝物質	C_1 [mM]	C_2 [mM]	C_3 [mM]
リン酸塩	≦40	≦5	≦2
炭酸塩	≦40	≦5	≦2
Mes/Tris	≦100	≦10	≦5
Hepes/Tris	≦100	≦10	≦5

[0015] In order to optimize the technique of this invention further, it is possible to choose the buffer which guarantees the optimum reaction condition according to the modality of interaction which combines an acceptor molecule with a precoat. For example, in the precoat containing streptoavidin, the pH domain of 5-9 is the optimum.

[0016] According to this invention, the combination of the buffer containing a volatile component like an acetic acid or a formic acid is also usable. However, in such combination of a buffer, after a volatile component's volatilizing and becoming the residue of a buffer, pH on a precoat may be changed greatly. It is because only one component of a buffer volatilizes and other components remain in a front face. Therefore, it is desirable to use the combination of the buffer which consists of only a volatile component or a nonvolatile component.

[0017] Again, in addition to the buffer matter, the washing solution used by this invention can be replaced with the buffer matter,

and can contain a surfactant. It assists that adding a surfactant in a washing solution removes a washing solution from a precoat, without leaving the residue substantially. As for especially a washing solution, it is desirable to contain a surfactant by the concentration below 0.005% (vol/vol) most preferably below 0.01% (vol/vol) still preferably below 0.02% (vol/vol) below 0.05 (the volume to full capacity ******)% (vol/vol). For the concentration of the surfactant in a washing solution, it is in the case below 0.001% (vol/vol) most preferably that the best result is obtained below 0.002% (vol/vol) still preferably below 0.003% (vol/vol). About some desirable surfactants, the desirable concentration C1, the desirable concentration C2, and the most desirable concentration C3 are especially shown in Table 2.

[0018]

[Table 2]

界面活性剤	C_1 [%(vol/vol)]	C_2 [%(vol/vol)]	C_3 [%(vol/vol)]
Tween 20	≦0.01	≦0.005	≦0.003
n-オクチルグルコシド	≦0.02	≦0.01	≦0.003
Chaps	≦0.01	≦0.005	≦0.003
Brj 35	≦0.005	≦0.002	≦0.001

[0019] All surfactants are fundamentally suitable to remove a washing solution, without leaving the residue substantially. It can be determined by the preliminary experiment of 2-3 according to the modality and technical conditions (for example, format of a reagent base material) of a coat whether a certain surfactant is suitable for a certain examination format if it is this contractor. Especially, the liquid applied behind must flow with a surfactant, or you have to pay attention so that only a few will not spread. From this ground, especially (related especially with operating concentration) when using a surfactant SDS, you have to pay attention.

[0020] The washing solution used by this invention can also contain again other additives which have an advantageous effect to the technique of this invention. The additive which has a stabilization effect to a precoat especially can be used. It is enabled to open a time interval in a manufacture process by this between pre coating of a reagent base material, and an application of the specific coat for each joint assays. A saccharide, salts, and a blocking reagent are mentioned as an example of such other additives. The desirable concentration C1 about these matter, the desirable concentration C2, and the most desirable concentration C3 are especially shown in Table 3.

[0021]

[Table 3]

物質	C_1 [% (wt/vol)]	C_2 [% (wt/vol)]	C_3 [% (wt/vol)]
糖類	≦0.5	≦0.1	≦0.05
塩類	≦0.5	≦0.1	≦0.05
ブロッキング試薬	≦0.2	≦0.02	≦0.005

[0022] The molecular weight of a desirable saccharide is the saccharide (for example, cane sugar) of 500 or less low molecular weight especially preferably 1000 or less. The sugar oligomer of the low molecular weight containing one, two, or three sugar units is the most desirable. The salts used preferably are salts which seldom form a crystal like a disodium tartrate and a dihydrate. As a blocking reagent, the protein of the low molecular weight like a peptone is used preferably.

[0023] The concentration of each matter in a washing solution can be adjusted according to the specific requirements for an examination, such as a modality of coat, and a size of an examination format. In order to attain suction which makes the amount of the possible residue the minimum in the case of the reagent base material which follows, for example, has a comparatively big reaction field (diameter:> 1cm), it is desirable to use the washing solution containing a pure water or the comparatively low-concentration above-mentioned matter (a buffer, a surfactant, other additives).

[0024] As for a drainage-system solution, it is desirable to pass quickly on a precoat, to remove completely next (for example, suction), and to make a precoat into a dryness. It is desirable to use the precoat which has a canal-property.

[0025] Next, the 2nd quart is applied to the washed precoat. It is applied on a reagent field as a zone where the domain was defined spatially including this acceptor molecule that can combine the 2nd quart with a precoat. As for an acceptor molecule, it is desirable to contain the 2nd joint partner of a joint pair who can do the interaction of for example, an immunological reaction, streptoavidin / avidin interaction, etc. to the 1st joint partner of a joint pair applied as a precoat by high compatibility, or can join together by covalent bond. It is got blocked, for example, streptoavidin or an avidin may be applied as a precoat, and an acceptor molecule contains a biotin component in that case. As for an acceptor molecule, it is desirable to make it combine with a precoat according to the equilibrium constant KM>=108 l/mol interaction of high compatibility. By the 2nd-quart application to the washed precoat, the examination zone of reproducible uniform profile and size can be obtained.

[0026] This effect becomes still clear with an attached drawing. Drawing 1 shows the miniaturization examination zone obtained by the conventional method of not performing a washing process. Drawing 2 shows the miniaturization examination zone obtained by the technique of this invention.

[0027] Drawing 3 is a thing explaining the effect of a washing process. a among drawing 3 What put water into Enzymun test

tube (polystyrene spool which is not coated) which is not coated is shown. Enzymun test tube (the polystyrene spool which coated streptoavidin --) with which b coated streptoavidin What put water into Boehringer Mannheim, order-number No.1144553, and "Enzymun-Test Streptavidin Tube" is shown. After coating c with streptoavidin, it shows what put water into Enzymun test tube washed and dried.

[0028] It is proved clearly that the spot profile of the advantage attained according to a washing process, i.e., the uniform examination zone which has repeatability on a reagent field, is obtained by comparison of the examination zone shown in the drawing 1 and the drawing 2 .

[0029] When water is put into Enzymun test tube which is not coated as shown in a of drawing 3 , a completely level water-air interface is formed. This means that **** of the wall surface by water is small. On the other hand, when water is put into Enzymun test tube pre coated with streptoavidin, the water-air interface which curved greatly as shown in b of drawing 3 arises. By pre coating, **** of a wall surface becomes large. When washing ****** of the test tube first pre coated with streptoavidin is violently carried out with a drainage-system solution, as shown in c of drawing 3 , a level water-air interface arises. A precoat shows a canal-property after a washing process and, for this reason, a wall surface does not get wet slightly merely in water.

[0030] According to this invention, the non-**** material of all solid-states can be used as a solid non-**** base material. As for this base material, what consists of tic [a metal, glass or / a press] preferably, and especially consists of polystyrene is desirable.

[0031] As for the 2nd quart, it is desirable to apply with the gestalt with a diameter of 10 micrometers - 10mm of the zone where the domain was defined spatially. Since especially the technique of this invention is suitable for production of a miniaturization examination format, as for the 2nd quart, it is desirable to apply preferably especially with the gestalt with a diameter of 20 micrometers - 200 micrometers of the zone where the domain was defined spatially the diameter of 10 micrometers - 500 micrometers. It is possible to perform the 2nd-quart application using well-known technique. It is [the 2nd quart] convenient to apply by using for example, a liquid jet process (liquid jet process) as a drainage-system solution with the gestalt of the minute drop of volume l of 0.00001-1micro. However, according to this invention, it is also possible to coat a microtiter plate and a big reaction container like a cuvette.

[0032] The whole field of the reagent field of a solid-state base material is coated, and it is desirable to apply the 2nd quart to the precoat of this whole field with the gestalt of the examination zone where the domain was defined spatially after that. However, it is also possible to apply a precoat beforehand with the gestalt of a spot. However, since the 2nd quart must be correctly arranged on this pre coated zone in this case, it will take time further.

[0033] Since the technique of this invention is reproducible, it is enabled to offer this examination system of the gestalt miniaturized so that the considerable reduction of the amount and analysis time of the examination system containing some identities or different examination zones, especially the announcer light needed could be carried out. Therefore, it is desirable to apply to a base material the zone which is the 2nd quart as which the domain was determined spatially [some]. All of these zones may contain the same acceptor molecule. In this case, it is possible to examine simultaneously two or more sorts of different samples on one examination base material. When the acceptor molecule from which at least two of these zones are different is included, it is possible to measure the announcer light of several sorts simultaneously in one sample. By such system, the thing for which the examination zone where the domain was defined clearly spatially is produced, and especially the thing for which every smearing of an acceptor molecule is prevented are important. It is enabled to ensure not bringing the result which contamination of the neighboring zone by the thing for which surely the announcer light of the purpose combinable with a specific examination zone is measured, and the acceptor molecule of other examination zones mistook only in such a case.

[0034] The precoat which could acquire still another theme of this invention by the above-mentioned technique, and was given on the solid-state base material, the solid phase which comes to contain the 2nd quart combined with this precoat with some gestalt of the circular zone where the domain was defined spatially -- it is -- this -- containing the acceptor molecule which the 2nd quart has combined with this precoat -- and -- this -- it is the above-mentioned solid phase characterized by for the diameter of the zone of the 2nd quart being 10 micrometers - 10mm, and the differentiation between each zone being less than 2.5% especially preferably less than 5% preferably less than 10% As for this solid phase, it is desirable that the diameter of an examination zone especially uses 10-500 micrometers by the 20-200-micrometer miniaturization examination system preferably.

[0035] Furthermore, it includes that such solid phase is used for this invention in joint assays, such as a thing to use for a detection of the announcer light of one sort or several sorts especially an immunoassay, and a nucleic-acid hybridization assay.

[0036] The process which still another theme of this invention is the technique for improving the homogeneity of a coating zone as which the domain was determined in between non-**** base material absentminded on a solid-state base material, and applies a precoat on the reagent field of (a) this solid-state base material, (b) the process which washes the base material which gave this precoat with a drainage-system solution, and the 2nd quart which can combine with (c) this precoat It is the above-mentioned technique characterized by including the process applied with the gestalt of the zone as which the domain was spatially determined on this reagent field on the base material which gave this precoat, and **.

[0037] The problem produced frequently [in case the solid phase for joint assays is manufactured and/or used] is the smearing of the acceptor molecule to the outside of the zone which is made into the purpose at the time of applying a re-coating solution and where the domain was defined spatially.

[0038] The 2nd mode of this invention therefore, the coating solution containing an acceptor molecule Are obtained by applying and processing to the predetermined field to which the domain of a solid-state base material was defined with a re-coating solution continuously. This coating solution is applied so that it may be the technique of preventing the smearing of the solid phase as which the domain was determined spatially and (a) this acceptor molecule may essentially join together quantitatively in

the predetermined field where the domain was defined, And it is related with the above-mentioned technique of coming to contain at least one of the meanses of preventing [the reunion of the acceptor molecule eluted by application of (b) after-treatment solution (re-coating solution) from the predetermined field where this domain was defined] **.
[0039] Especially, by [which should be surprised] using at least one of the meanses of these, the smearing of the solid phase as which the domain was determined spatially could be prevented, and it became clear that the examination zone which has the profile and size which are reproducible as the result and where the domain was defined clearly is obtained. This technique is monolayer coating] usable also to the multilayer coating tip.
[0040] An acceptor molecule may usually be applied to a solid-state base material with the gestalt of a drop small as a drainage-system solution, and this base material may be pre coated by the case. Next, an acceptor molecule is diffused from this solution to this solid-state base material or a precoat, and is combined with this. This joint interaction may be an interaction of high compatibility like an immunological reaction, or streptoavidin / avidin-biotin interaction, or may be covalent bond. By the usual solid phase system of the conventional technique, when the experiment for analyzing for example, an announcer light is conducted according to the process which continues after a coat application, or when a re-coating solution is applied, the elution of the molecule which has not been combined pertinently uncombined from this solid phase happens.
[0041] The solid phase coated specifically is processed with the re-coating solution which has the function which blocks an un-specific bonding site and usually stabilizes an acceptor molecule to the influence from the exterior. The ground this is needed is that the examination zone on the reagent field of solid phase is not usually occupied 100% completely. However, the free fraction which remains on the base material or the precoat corresponds to the un-unique bonding site which other sample components other than the target announcer light can combine.
[0042] Furthermore, the coat of an acceptor molecule usually also contains the trauma molecule of slight proportion, and other sample components other than the target announcer light can combine it there in un-unique. Such a site is blocked by applying the re-coating solution containing for example, inactive protein, and, in this way, a sample component can combine it no longer later during analysis. Furthermore, a re-coating solution is useful also to stabilizing an acceptor molecule or coating to the influence of external (for example, temperature and humidity) in many cases. For example, in many embodiments of an analysis system like a microtiter plate or ES tube, operation in which a re-coating solution achieves a blocking operation and a stabilization effect exists.

[0043] Although the re-coating solution needs to be used for many examination formats, this is accompanied by the smearing of the acceptor molecule applied previously simultaneously. If a re-coating solution is applied, the elution of the acceptor molecule which has not been combined pertinently uncombined will happen. The regrettable smearing of the solid phase as which the domain was determined especially spatially beforehand combinable [these elution acceptor molecules] with the still combinable site (accessible sites) of a solid-state base material or a precoat therefore arises. An acceptor molecule is because it can adhere besides the 1st application section. Such a smearing is a serious fault when the zone of some containing an acceptor molecule especially different, respectively where the domain was defined spatially is applied to a base material. Since elution of an acceptor molecule and combination of the elution acceptor molecule to other sites of solid phase imitate mixture of the acceptor in an examination zone and may come, they become unable to obtain a clear analysis result. This problem is produced especially in a miniaturization examination format. Also in the examination for which a re-coating solution is not used at all, the same problem arises at the time of the last which applied the sample solution.
[0044] It became clear that it is possible to prevent the smearing of the solid phase as which the domain was determined spatially by now applying the coating solution which should be surprised and which especially essentially causes combination of a quantitive acceptor molecule in a predetermined zone. Since an acceptor molecule joins together about 100%, the elution of this acceptor molecule can be prevented. Many especially more preferably [it is desirable and] than 90%, from 95%, there are many acceptor molecules and they are combined. [than 99%] [more / it is the most desirable and] As for a quantitive combination of an acceptor molecule, it is essentially desirable to be attained by using the coating solution which has acceptor molecule concentration lower than sufficient threshold value concentration to occupy a predetermined zone completely. When a coating solution contains an acceptor molecule fewer than the amount which can be combined with an examination zone, all the applied acceptor molecules are combined with an examination zone, it becomes behind and the uncombined acceptor molecule or the acceptor molecule which has not been combined pertinently which may be eluted (for example, re-coating solution) does not exist. The threshold value concentration about various kinds of acceptor molecules is shown in Table 4.
[0045]
[Table 4]
表4：各種コーティング分子の限界濃度

受容体分子	直径 10μm	直径 100μm	直径 1 mm
抗体	2.5 mg/ml	250μg/ml	25μg/ml
抗原（例えばp24）	600μg/ml	60μg/ml	6μg/ml
ペプチド(MW 3000)	150μg/ml	15μg/ml	1.5μg/ml

[0046] It is desirable to use the coating solution containing the acceptor molecule of concentration (especially [Less than 50% / Preferably / of a threshold value concentration] good **** less than 25%) lower than a threshold value concentration.

[0047] furthermore, not the acceptor molecule combined powerfully, for example, a monomer antibody, but bridge formation -- it is desirable to use an antibody It is possible to prevent a smearing by making an antibody construct a bridge and forming the polymer (polymer) of molecular weight bigger than 1,200,000 Dalton especially. The protein with which the ground constructed the bridge highly is because it became clear to combine with a solid-state base material (especially plastics front face) very well. The tBSA conjugate like the recombination field HBc antigen which carries out self-flocculation and forms the macromolecule of the domain of several 1 million Dalton and tBSA-biotin, tBSA-streptoavidin, or tBSA-anti--TSH antibody is especially used preferably as an acceptor molecule. It became clear the thing which an acceptor molecule combines about 100% in all cases, therefore that the smearing of an acceptor molecule does not arise at all on the outside of the zone made into the purpose.
[0048] You may apply an acceptor molecule to the precoat which may apply to a solid-state base material directly, or was applied to the solid-state base material. It is desirable to use the acceptor molecule which has 108 l/mol high joint compatibility at least to this precoat in the case of a precoat.
[0049] Furthermore, that it can prevent by adding a elution retarder in a coating solution made clear the smearing of the solid phase as which the domain was determined spatially. A elution retarder forms a coat (paint film) on an examination zone, after a coating solution dries, and it is understood to be the matter by which the direct elution of the uncombined acceptor molecule at the time of adding a re-coating solution or a sample solution or the acceptor molecule which has not been combined pertinently is prevented or delayed. a suitable elution retarder is like cane sugar, PVP (polyvinyl pyrrolidone), or a dextran -- molecular weight is the saccharide of 10,000 to 100,000 Dalton preferably Other suitable elution retarders are gelatin and a cellulosic, and when an acceptor molecule reacts with the precoat applied previously, especially being used preferably is a methyl cellulose. Addition of a elution retarder delays the elution of an uncombined acceptor molecule until it is also probed that it is blocked by the means with all the free sites suitable at least on a solid-state base material, and a elution acceptor molecule already joins together and it is lost.

[0050] In the 2nd mode, the reunion of the acceptor molecule eluted from the predetermined field is prevented by applying a re-coating solution. This is preferably attained by adding the blocking matter in a re-coating solution. In this case, it is important to ensure to restrict and to join together quickly with the possible blocking matter. This can be preferably attained 0.5% of the weight or more by adding 1% of the weight or more of the high-concentration blocking matter (bovine serum albumin), for example, 1% of the weight of BSA, protein C, casein, etc. in a re-coating solution. Still suitable blocking matter is a peptone, cow IgG, lagomorph IgG, a collagen, gelatin, a polyethylene glycol, and a surfactant (for example, Tween 20).
[0051] The joint speed of the blocking matter (especially blocking protein) is improvable again with a suitable buffer additive like MgCl2 3%. In applying to a precoat rather than applying a coating solution to a solid-state base material directly, it chooses the blocking matter which reacts specifically with this precoat. In addition to it instead of [like blocking protein] the un-specific blocking matter, the blocking effect is further improvable by using the combinable specific blocking matter for a precoat specifically. In this case, the solution containing a biotin-ized acceptor molecule is applied for example, to a streptoavidin precoat. A biotin solution is applied to this after xeransis, and a still free streptoavidin bonding site is still saturated.
[0052] It is also possible to prevent the smearing of the solid phase as which the domain was spatially determined by applying a re-coating solution for a short time so that the eluted acceptor molecule may be thinned powerfully immediately according to this invention finally. the longest time about this -- usually -- they are 50 mses especially preferably 250 mses preferably 500 mses In this case, it is advantageous that quick mixture is ensured and the field where a elution acceptor molecule becomes high concentration locally in a re-coating solution is made not to be generated. Efficient mixture can attain for example, a re-coating solution by making certainly into the minimum the contact time of the possible re-coating solution which restricts, applies by the early rate of flow, and is possible, and an examination zone.
[0053] Especially, it uses preferably combining some in the above-mentioned means, and the eluted acceptor molecule is made not to join out of the target examination zone together. A elution retarder is added in a coating solution, the blocking matter is added in a re-coating solution, and it is clear that especially the thing that quick mixture of a re-coating solution is ensured simultaneously with it is advantageous.
[0054] Since especially this technique is advantageous in the miniaturization examination format, as for the solid phase as which the domain was determined spatially, it is desirable that 10-500 micrometers of diameters are 20-200 micrometers especially preferably.
[0055] this invention relates to the solid phase acquired by the above-mentioned technique for preventing the smearing of the solid phase as which the domain was determined spatially again. this solid phase It is manufactured by applying and processing the coating solution containing an acceptor molecule to the predetermined field of a solid-state base material with a re-coating solution continuously, and sets there. This acceptor molecule is arranged in the zone where the domain was defined spatially, and it is characterized by existing 90% or more 80% or more in the zone of this acceptor molecule where the domain was defined especially in [it is / 95% or more of / especially desirable, and] this space. Such solid phase makes possible the analysis result in which a reliability has repeatability highly. This kind of solid phase is usable to the detection of the announcer light of one sort or several sorts.
[0056] Many another causes of a result that the mistake was able to be made in in a joint assay are combination of sample components other than the announcer light of the purpose to the solid phase which coated an un-unique combination, i.e., streptoavidin, or the avidin. Therefore, this invention is the technique for reducing again the un-unique combination to the solid phase which coated streptoavidin or the avidin, and relates to the above-mentioned technique containing the process which applies the conjugate which consists of a (a) macromolecule and a biotin, and the process which applies (b) monomer

streptoavidin or an avidin to solid phase.
[0057] It made this technique clear especially that the considerable reduction of the un-unique combination to the solid phase which should be surprised and which coated streptoavidin or the avidin can be carried out. By using monomer streptoavidin, the considerable reduction of the un-unique combination of the conjugate to solid phase and other sample components is carried out especially.
[0058] The conjugate which consists of the macromolecule and biotin like tBSA preferably is applied to a solid-state base material with the gestalt of a minute drop as a drainage-system solution. In this case, it is desirable that a macromolecule and a biotin use 1:1-1:3, and the conjugate that becomes by the proportion of 1:1-1:2 preferably especially. Thus, if the coating protein of the few amount of biotin-ized theory is used, it will be enabled to reduce an un-unique combination of especially conjugate further much more.
[0059] The diameter of solid phase is especially preferably [especially] suitable to the miniaturization examination format of 5mm or less for the technique of this invention for reducing the un-specific combination to the solid phase which coated streptoavidin or the avidin 10mm or less preferably again. Further, another theme is solid phase acquired by the above-mentioned technique of this invention, and this is characterized by having the layer of the monomer streptoavidin given on the precoat of the conjugate which consists of a macromolecule and a biotin, and this precoat, or an avidin. Such solid phase [especially] is used for a detection of an announcer light. The following examples explain this invention further.

[Translation done.]

EXAMPLE

[Example] The production examination zone of the spot to the front face which coated [example 1] streptoavidin was coated with the coating solution containing amount compound BSA[of macromolecules]-streptoavidin (particle size : about 100nm). The coating solution was attracted after the reaction time for 15 minutes, and the examination zone was blocked with NaCl, 0.2% cane sugar, and the solution that contains BSA 0.05% 0.05%. In the examination zone shown in drawing 1 , on the other hand, using the drainage-system medium, after incubating for 5 minutes, the suction xeransis of the precoat was only carried out, it carried out, and the suction xeransis of the washing process by this invention was carried out continuously in the examination zone shown in drawing 2 .

[0061] [Example 2] 3.7g Tris [a tris (hydroxymethyl) aminomethane] and 4.75g MES (2-morpholino ethane sulfonic acid) were melted in 5l. water. 5ml of this washing buffer solution was poured and washed in the pre coated reaction container (nominal capacity:50microl). Then, the suction xeransis of this reaction container was carried out using the suction needle (aspiration needles) of one piece or some. By applying the 2nd quart on this washing and the dry precoat, an examination zone which is shown in drawing 2 and where the domain was defined narrowly spatially was obtained.

[0062] The [example 3] streptoavidin precoat was applied on the solid-state base material. On this coat, the coating solution containing a mono-biotin-ized antibody was applied with the gestalt of a small drop. After drying this drop, the solution which contains a 3mg [/ml] biotin in 50mM K2HPO4 was applied to this. This biotin solution was removed after several seconds, the solution which contains BSA (bovine serum albumin) and 2% cane sugar 1% was applied, and this was also removed after several seconds. Then, this system was dried. Thus, the solid phase without the smearing of the acceptor molecule in the outside of the target reaction field as which the domain was determined clearly spatially was acquired.

[0063] [Example 4] It investigated about the un-specific combination on various kinds of streptoavidin solid phase. A result is shown in Table 5.

[0064]

[Table 5]

コートの種類	TSH コンジュゲート	p24 コンジュゲート	ヒト IgG
tBSA－ストレプトアビジン	24	31	105
tBSA－ビオチン ＋ 多量体ストレプトアビジン	3	71	63
tBSA－ビオチン ＋ 単量体ストレプトアビジン	0	4	38

[0065] An un-unique combination is considerably reduced with the combination of the conjugate and monomer streptoavidin by this invention so that clearly from the above.

[0066] The same peptide of [example 5] direct coating **** [some] was combined with BSA, and the poly-hapten was obtained. This poly-hapten was melted by 50microg [/ml] concentration into the buffer solution containing 5mM Mes, 5mM Tris, and 1% cane sugar. Then, the drop of volume 150pl was applied to the base material made from polystyrene, and it incubated for about 5 seconds, and dried for about 60 seconds. Then, the blocking solution which contains BSA and 2% cane sugar 1% was applied, and it incubated for 10 seconds. Then, this solution was attracted and the blocking solution which contains BSA and 2% cane sugar 1% was added. Then, this solution was attracted and it dried.

[0067] Pre coating **** of [example 6] streptoavidin and the biotin-ized macromolecule (for example, heat flocculation BSA) were applied to the base material made from polystyrene (nominal capacity:50microl) by 100microg [/ml] (inside of PBS) concentration, and it incubated for 5 minutes. Then, this was attracted and the streptoavidin solution of 100microg [/ml] (inside of PBS) concentration was added. After incubating for 5 minutes, the washing process was performed, and the suction xeransis of this solution was carried out continuously.

[0068] Various kinds of biotin-ized DNA usurpation probes (18-mer) were melted in 5mM Mes, 5mM Tris, 1% cane sugar, and the buffer solution that contains 0.5mg /of BSAs ml by the concentration of 1microM. Then, the drop of volume abbreviation 150pl was applied to the configuration of an array at the base material made from polystyrene. After having incubated for about 5 seconds and drying for about 60 seconds, in the 50mM phosphoric-acid 2 potassium, the solution containing a 3mg [/ml] biotin

was added, and it incubated for 2 seconds. Then, this solution was attracted and the blocking solution which contains BSA and 2% cane sugar 1% was added, and this solution was attracted and it dried.
[0069] Pre coating PAB<mouse-Fcgamma> S IgG by the [example 7] **-mouse antibody was melted in the buffer solution (pH 7.4) which contains 40mM potassium phosphate in 0.9% sodium chloride by 30microg [/ml] concentration. This solution was added to the reaction container (nominal capacity:50microl). After having carried out the incubation for 15 minutes and drawing in continuously, the blocking solution which contains BSA and 2% cane sugar 1% was added. After incubating for 5 minutes, the washing process was performed according to this invention, and the suction xeransis of this solution was carried out continuously.

[0070] Fusion protein (mouse-Fcgamma+CD28) was melted in 5mM Mes, 5mM Tris, 1% cane sugar, and the buffer solution that contains 0.5mg /of BSAs ml by 100microg [/ml] concentration. It applied to the base material made from polystyrene which the above [the drop of volume abbreviation 150pl] coated, and incubated for about 5 seconds. After drying for about 60 seconds, 50mM potassium phosphate buffer solution (pH 7.4), the 100microg [/ml] mouse IgG, and the blocking solution that contains 1mg /of BSAs ml were added. After incubating for 10 seconds, this solution was attracted and the blocking solution which contains BSA and 2% cane sugar 1% was added, and this solution was attracted and it dried.
[0071] The macromolecule with a particle size [containing [example 8] universal streptoavidin examination zone BSA and streptoavidin] of about 100nm was melted in the solution containing 20mM phosphate buffer solution (pH 7.4) and 1% cane sugar by 500microg [/ml] concentration. The drop of volume abbreviation 150pl was applied to the base material made from polystyrene, and it incubated for about 5 seconds, and dried for about 60 seconds. Then, it added, and it incubated for about 10 seconds and the solution which contains BSA and 2% cane sugar 1% was attracted. Next, it added, and it incubated for 20 minutes and the solution which contains BSA, 2% cane sugar, and 1microg [/ml] biotin-ized anti--TSH antibody 1% was attracted. Finally the blocking solution which contains BSA and 2% cane sugar 1% was added and attracted, and it dried. Consequently, very precise miniaturization TSH specific solid phase was acquired.
[0072] By 100microg [/ml] (inside of PBS) concentration, it applied to the base material made from polystyrene (nominal capacity:50microl), and it incubated for 5 minutes and the examination zone biotin-ized macromolecule for [example 9] **-German measles antibodies (for example, heat flocculation BSA) was attracted. Then, the streptoavidin solution of 100microg [/ml] (inside of PBS) concentration was added. After incubating for 5 minutes, the washing process was performed according to this invention, and the suction xeransis of this solution was carried out continuously.
[0073] The biotin-ized **-German measles antibody was melted in the buffer solution containing 5mM Mes, 5mM Tris, 1% cane sugar, and 0.5mg [/ml] BSA by 50microg [/ml] concentration. The drop of volume abbreviation 150pl was applied to the above-mentioned base material. It incubated for about 5 seconds and this was dried for about 60 seconds. Then, in the 50mM phosphoric-acid 2 potassium, the solution containing 3mg [/ml] biotin was added, it incubated for about 2 seconds, and this solution was attracted. Next, 0.1%, it added, and it incubated for 20 minutes and Tween 20, and BSA and the solution which contains a 3U/ml virus (rubella virus) lysate in PBS were attracted 1%. Then, the blocking solution which contains BSA and 2% cane sugar 1% was added and attracted, and it dried.

[Translation done.]

* NOTICES *

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The miniaturization examination zone obtained by the conventional method of not performing a washing process is shown.
[Drawing 2] The miniaturization examination zone obtained by the technique of this invention is shown.
[Drawing 3] It is a photograph explaining the effect of the washing process of this invention.

[Translation done.]

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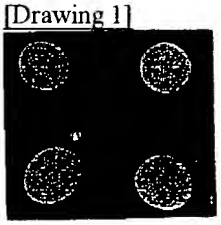
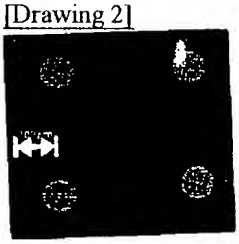
DRAWINGS

[Drawing 1]



[Drawing 2]



[Drawing 3]

[Translation done.]